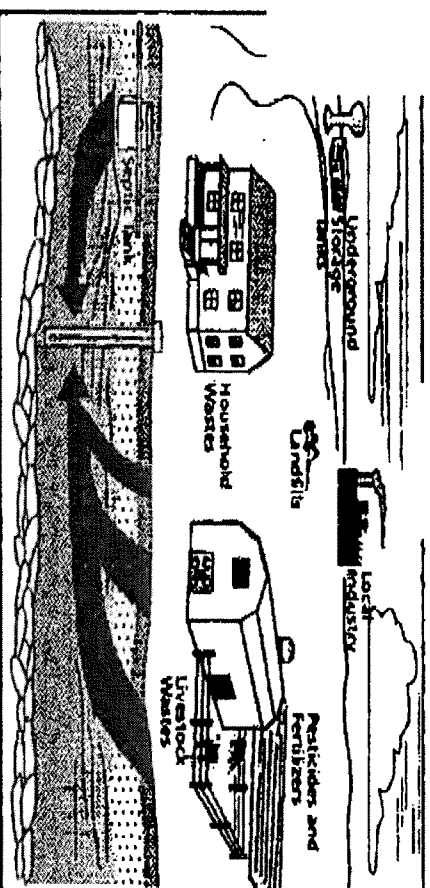


Possible Sources of Bacterial Groundwater Contamination

□ There are only two possible sources of *E. coli*, which is produced in the intestines of warm-blooded animals:

- *Human Waste*
- *Animal Waste*

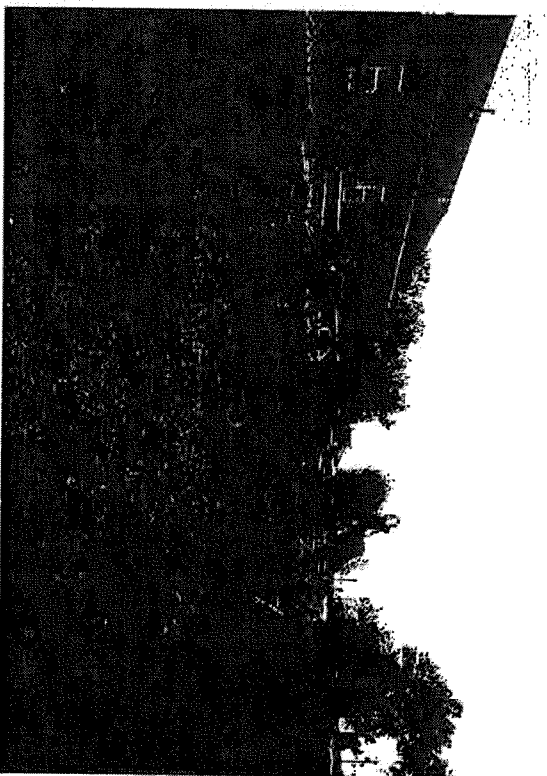


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Human Waste Contribution

- ▣ DEQ has investigated potential human waste contribution to Country Cottage well contamination.
- ▣ Two possible sources: septic and sewer.



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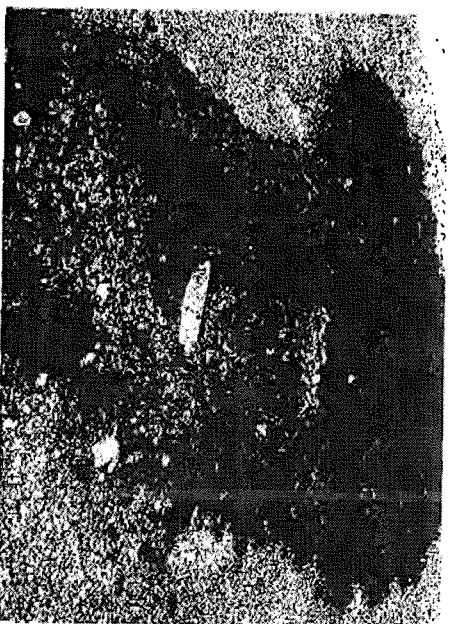


Photos from DEQ investigation on Nov. 5, 2008 14

Human Waste Contribution - Septic

- Abandoned septic system at Country Cottage located, lateral lines investigated. DEQ and Dr. Bert Fisher observe as follows: "dry hole, dry line, no biomass discoloration."
- Septic tank next door to Country Cottage inspected. Out of service for many years.
- **Conclusion:** Abandoned septic systems in the immediate vicinity of the Country Cottage well have been out of use for years and are not contributing any human waste to the groundwater.

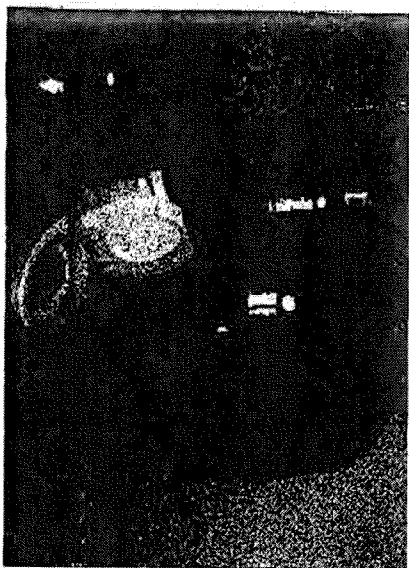
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Photos from DEQ investigation on Nov. 5, 2008

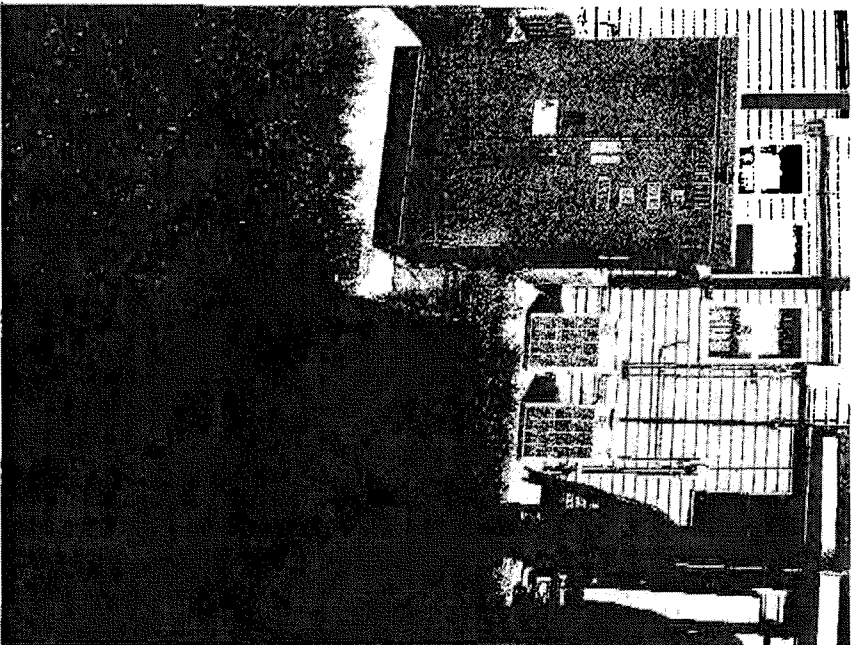
Human Waste Contribution - Sewer

- Nov. 5 - DEQ inspected the sewer connection to the Country Cottage with a dye test.
- Nov. 20 & Dec. 4 - DEQ inspected the sewer connection with a smoke test.
- **Conclusion:** Human waste from the sewer is not contaminating groundwater in the vicinity of the Country Cottage.



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Conclusion: Human Waste Contribution



Dr. Bert Fisher and Rick Austin, DEQ, investigate possible human waste contribution.

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□ Conclusions of Dr. Fisher:

- Animal Source: It is highly unlikely that the bacteria contamination in the Country Cottage well resulted from human waste. It is very likely, therefore, that this contamination resulted from animal waste.
- There is no proximate source of human waste that could have contaminated the well.
- Common Source: The large area of bacteria contamination suggests a large source, and a common source.
- The decrease in the concentrations of bacteria over time (August through November) suggest one or more upgradient events in the spring or summer as the cause of the bacteria contamination.

Animal Waste Contribution

- Two animal populations in the vicinity: cattle and poultry.
- Cattle populations are relatively small and dispersed. There are no concentrated cattle feeding lots or dairies in a ten mile radius.
- Poultry operations are large and concentrated.



Tyson poultry houses, 2.7 miles up gradient of Country Cottage - Terri Hughes, Grower for Tyson

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Animal Waste Contribution - Poultry

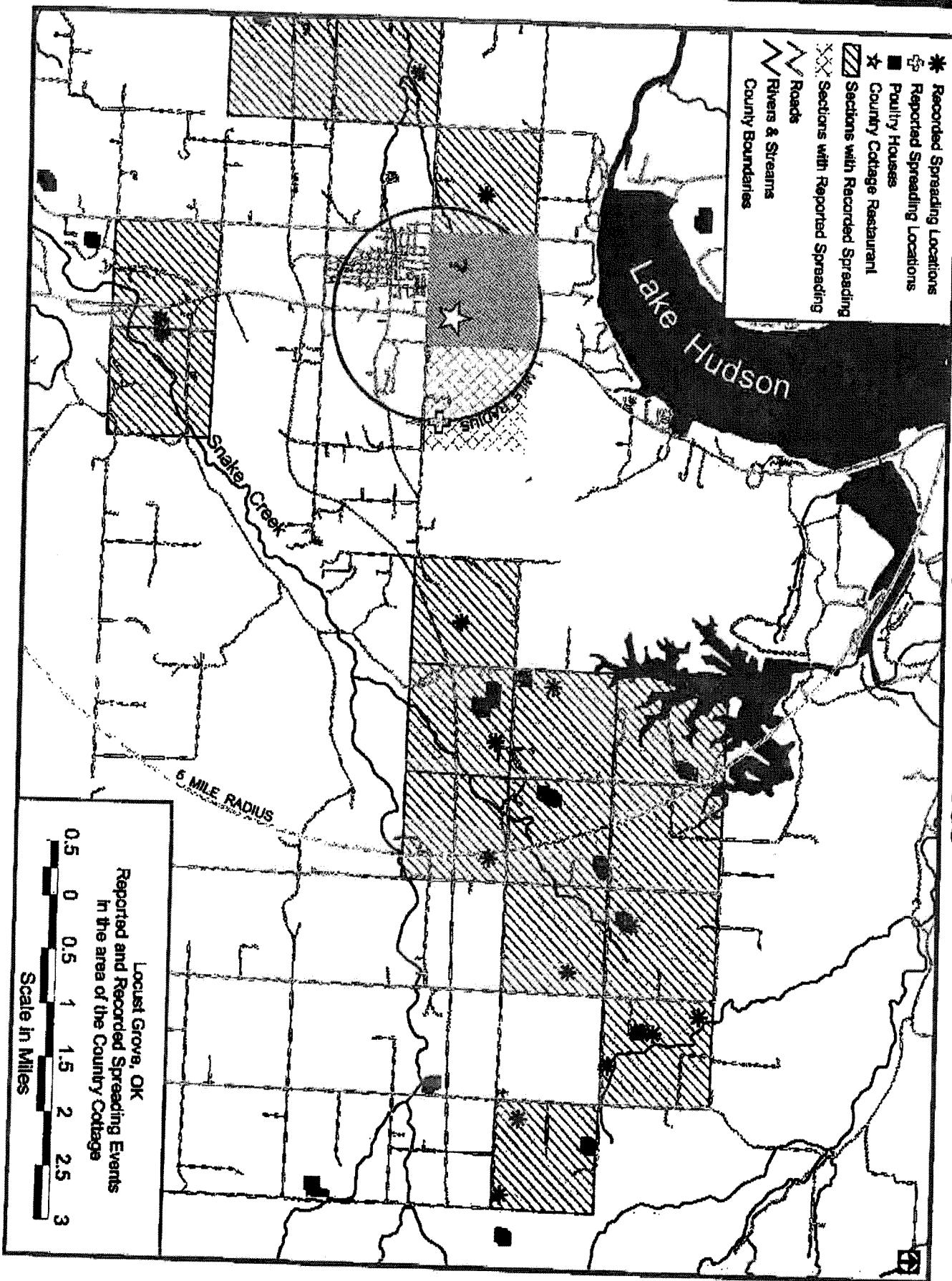


Examples of nearby active chicken houses (within 5 miles, NAIP aerial photos, 2008)

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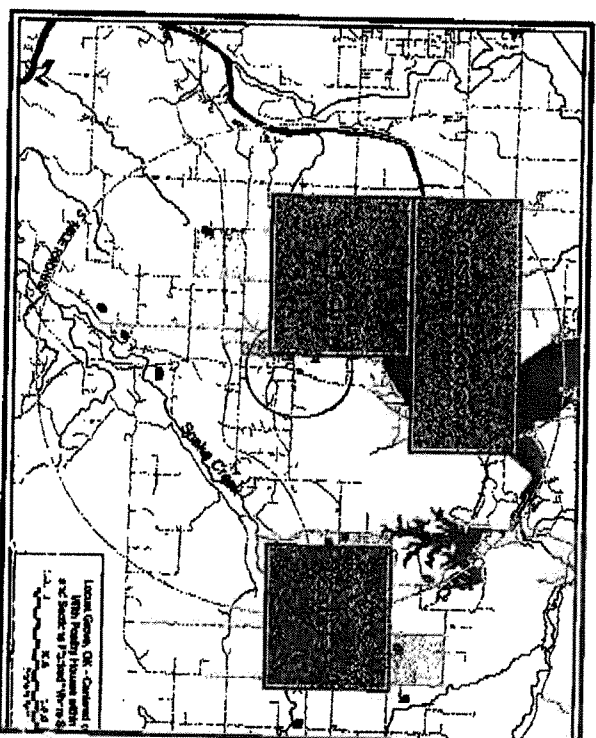
- Five-Mile Radius:
 - 39 active poultry houses.
 - 4 to 6 million birds produced a year.
 - 5,000 to 7,000 tons of poultry waste generated each year.
- Within 10 miles of Country Cottage well:
 - 113 active houses within 10 miles.

Poultry Houses and Litter Application Sites

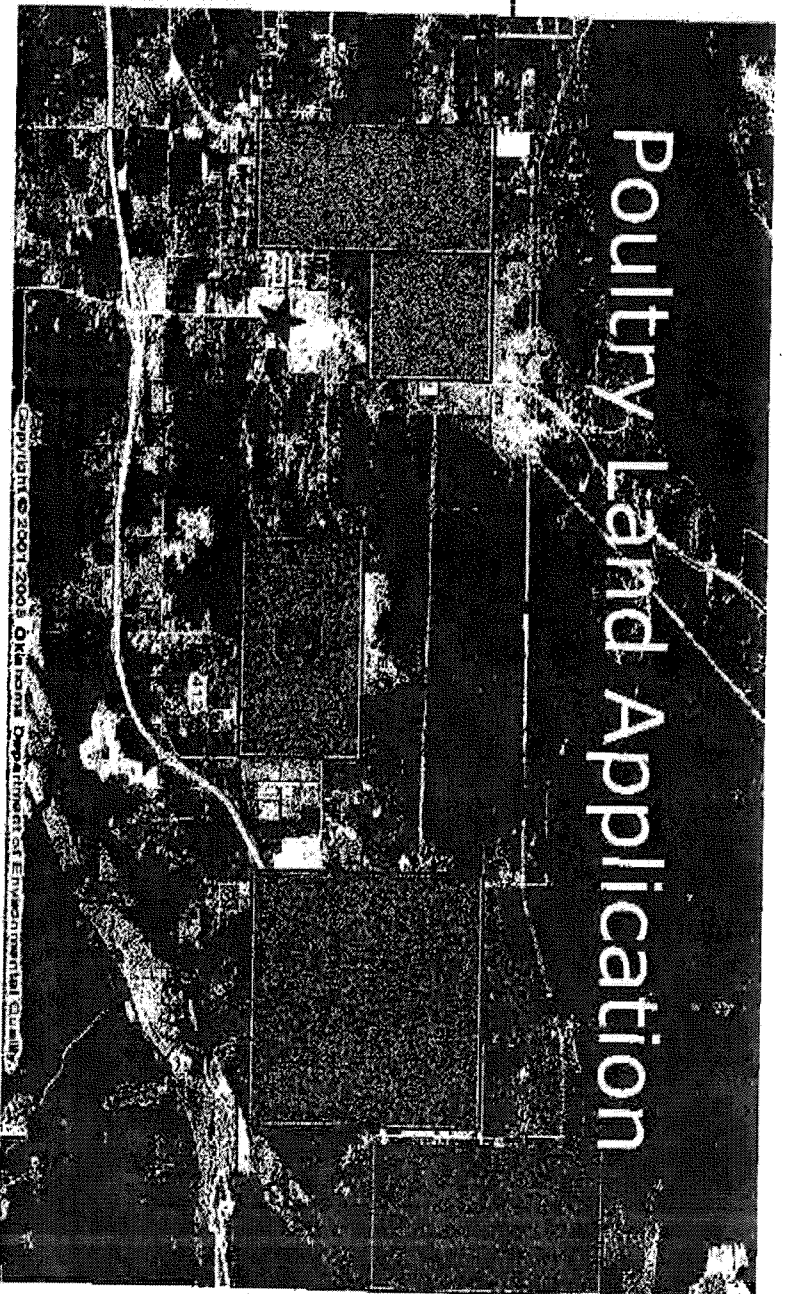


Animal Waste Contribution - Poultry

- Land application is documented near Hughes Tyson operation (2.7 miles) and on Perkins property (less than 2 miles) (ODAFF records).
- Possible land application within ¼ mile of Country Cottage at Eugene Koelsch property.



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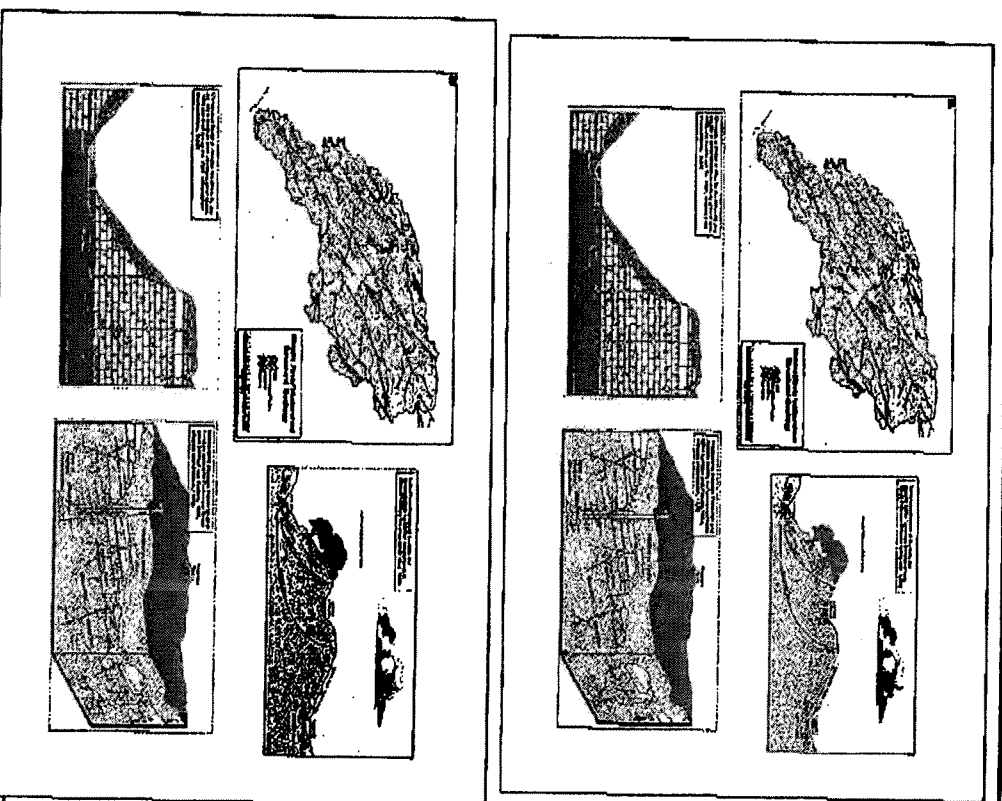
- Land application documented on Area "A" - Eugene Koelsch property. Approximately 1/4 mile from well. Location undetermined because Koelsch family owns property all around restaurant.
- Conflicting reports concerning land application on Connor property.
- Visual inspection by Dr. Bert Fisher confirms that Areas "A" "B" "C" & "D" all appear to be fields typically applied with poultry litter. (Monospecific, hay production, etc.) Confirmed by visual observation and historic aerial photos.
- Records from Perkins (applicator) confirm frequent and reoccurring land application on Area "C."
- ODAFF documents Area "D" is a confirmed land application area (Hughes and Cole).

Animal Waste Contribution - Poultry

□ According to Dr. Bert Fisher, the vicinity of Country Cottage restaurant is a mantled karst terrain, allowing for the easy transport of contaminants from the land to the groundwater.

□ Dr. Fisher believes that a contaminant pathway exists connecting the land-applied fields to the Country Cottage well.

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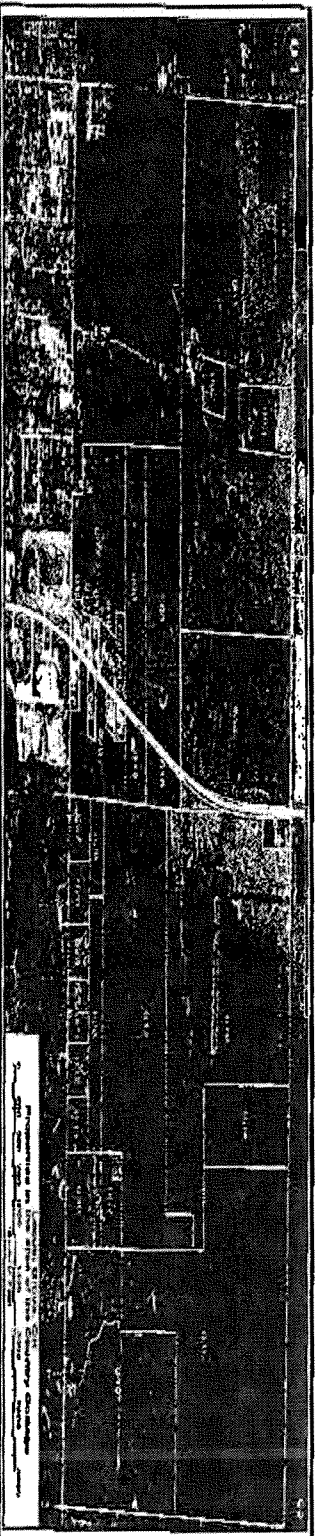


Animal Waste Contribution - PCA

- ▣ Dr. Roger Olsen performed a Principal Component Analysis (PCA) on water samples from several sites around and including the Country Cottage restaurant.
- ▣ The PCA analysis revealed a unique "chicken signature" at more than one location, including the well at the Country Cottage restaurant.
- ▣ Cattle signature and human waste signature "not present."

Country Cottage
Well "strong
chicken signature"
Two Samples

Newcomb well
"strong chicken
signature"



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Football Field
"weak chicken
signature"

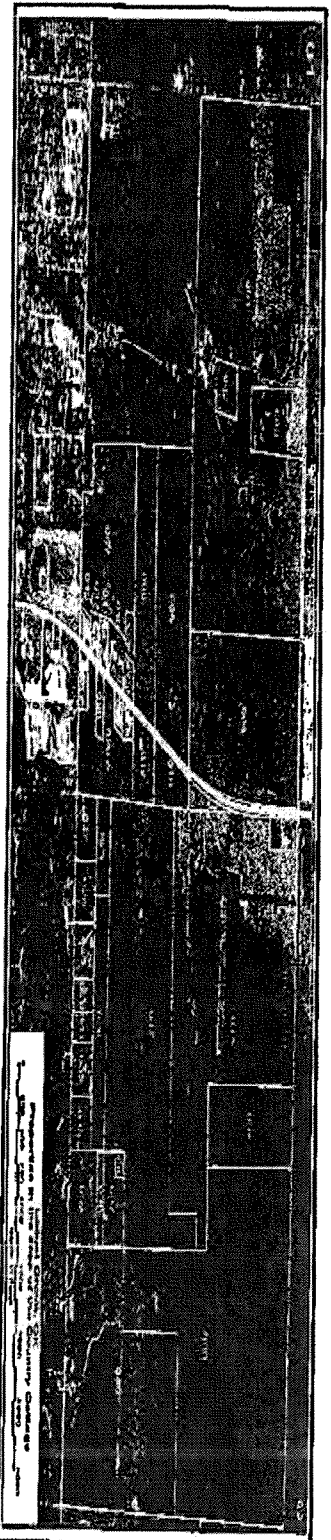
Animal Waste Contribution - PCR

- ▣ Northwind Laboratory (in consultation with Dr. Jody Harwood) conducted a DNA analysis of water samples from several wells (qPCR).
- ▣ Results show the presence of poultry-specific biomarker (*brevibacteria*) in several wells, including the country cottage.

Haddock Well
biomarker
PRESENT

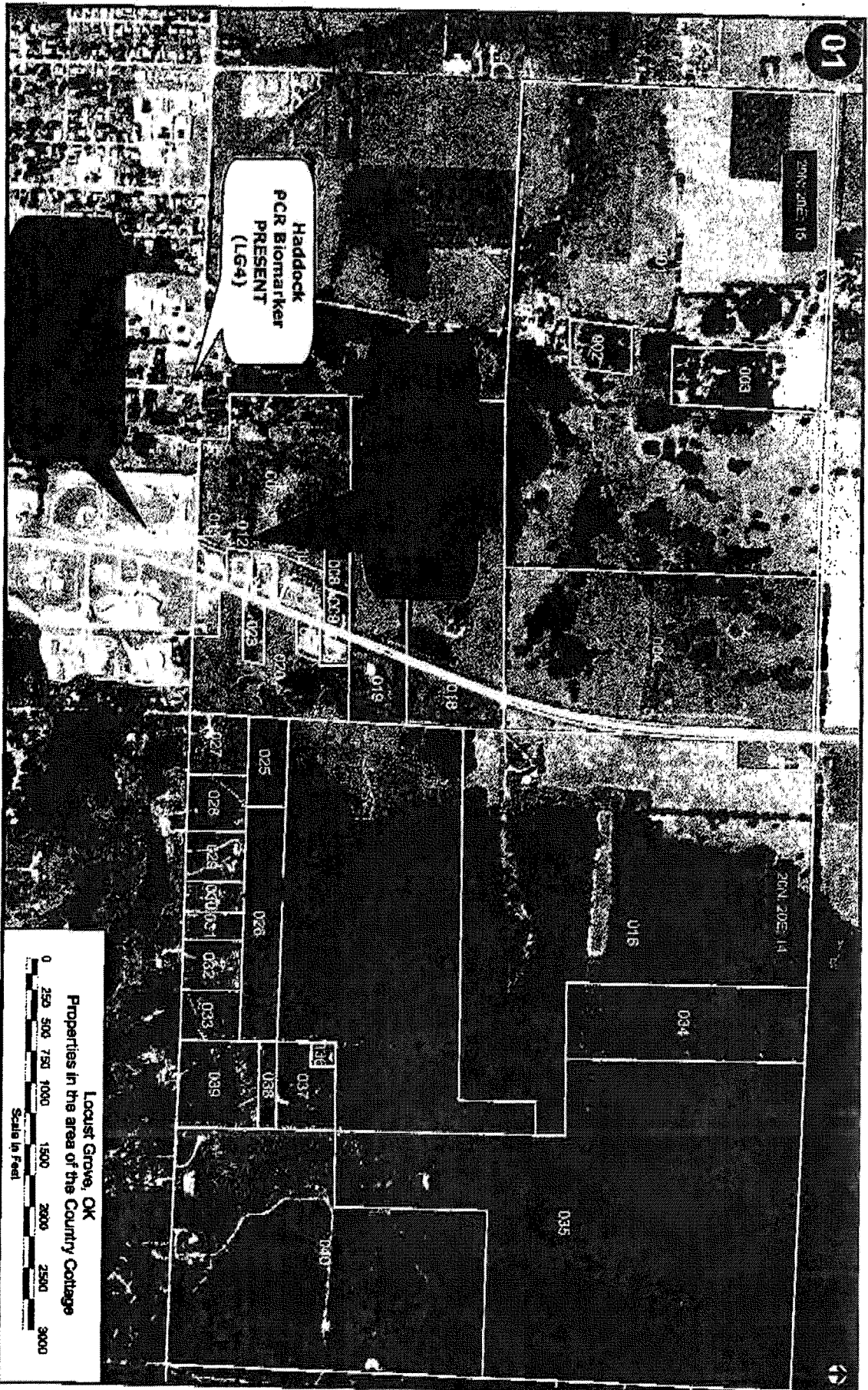
Country Cottage Well
biomarker
PRESENT

Football Field Well
Biomarker
PRESENT



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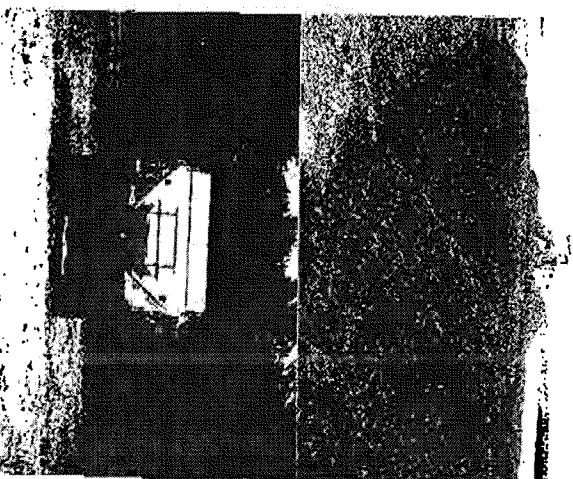
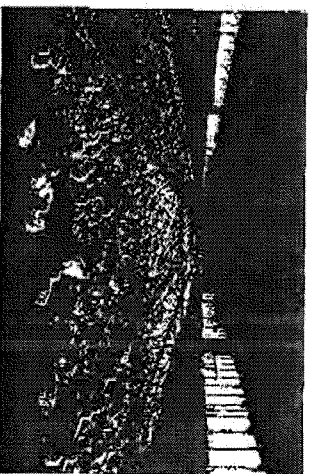
Summary of PCA and PCR Results



Summary

- The Country Cottage well is a possible source of the *E. coli* that caused the outbreak of illnesses.
- It is very unlikely that human waste contributed the *E. coli* in the Country Cottage well.
- A large "plume" of bacterial contamination in the groundwater is suggestive of a large source.
- Although cattle are present in the vicinity of the well, they are not confined or concentrated in large numbers.
- A significant poultry population is raised within a 5-mile radius.
- Poultry waste is land applied within the vicinity of the Country Cottage.
- The mantled karst terrain with a gradient from the pastures to the Country Cottage provides a pathway for bacterial transport.
- PCA results indicate that the "chicken signature" is present in the Country Cottage well, and other wells in the vicinity.
- PCR results prove existence of poultry-specific biomarker in groundwater of Country Cottage well, and other wells in the vicinity.

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Conclusion

- The likely source of the bacteria in the Country Cottage well is poultry waste.
- This poultry waste is a possible cause of the *E. coli* outbreak.
- The specific strain of *E. coli* that has caused illness and death, "O111", has been found in poultry waste.

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